

### AMENDMENTS TO THE SPECIFICATION

Please delete the Sequence Listing previously filed in this application and replace with the Sequence Listing text file submitted herewith *via* EFS-Web. Applicants specifically request and direct that this Replacement Sequence Listing be entered into this application

In the specification at page 1, after the heading and paragraph entitled "Related Applications" that was inserted by the First Preliminary Amendment, please insert the following new heading and paragraph:

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#### SEQUENCE LISTING SUBMISSION

The Sequence Listing associated with this application is filed in electronic format *via* EFS-Web and hereby incorporated by reference in its entirety into the specification. The name of the text file containing the Sequence Listing is Replacement\_Sequence\_List\_13744\_00021. The size of the text file is 118.5 KB, and the text file was created on May 13, 2009.

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Please amend paragraph [0013] of the English-language translation of the Specification as follows:

[0013] The application WO 02/097064 A1 (EP 1391502 A1) relates to microorganisms, in which the genes from the stages II, III, IV or V of the sporulation have been deleted or inactivated. They concern the genes *sigE*, *sigF*, *spoIIE*, *spoIISB* and *sigG* of *B. subtilis*, which reside within the locus of *spoIVCB* to *spoIIIC* of *B. Subtilis*. Using the databank SubtiList (~~available on~~ <http://genolist.pasteur.fr/SubtiList/genome.cgi> Web server (provided by Institut Pasteur, Paris, France), this can be narrowed down to the region of the positions from *ca.* 2 642 000 kb to *ca.* 2 700 000 kb of the total genome of *B. subtilis*, which has since become known. The object of this application was based on the elimination of superfluous or harmful activities of *baeillus* *Bacillus*

strains in order to improve the biotechnological production. By modifying the middle to late sporulation genes in this way, the use of the strains in question represses spore formation for the biotechnological production; this would have an advantageous effect on the nutrient utilization and energy utilization; the fermentation time could be simultaneously increased, thereby increasing the total yield of interesting valuable products.

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Please amend paragraphs [0030] and [0031] of the English-language translation of the Specification as follows:

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[0030] The amino [[acid-]] acid and nucleotide sequences given in ~~SEQ ID No. 2~~ SEQ ID NOs: 2 and 1 are those for RecA. All positions from 1 to 1047 encode for the protein; the last three represent the stop codon. They are designated as the gene and protein *recA* respectively RecA. They originate from the strain *Bacillus licheniformis*, deposited under the number DSM 13 at the Deutschen Sammlung von Mikroorganismen und Zellkulturen GmbH, (German Collection of Microorganisms and Cell Cultures) Mascheroder Weg 1b, 38124 Braunschweig (~~http://www.dsmz.de~~). Inventive solutions to the problem are represented by all factors or nucleic acids that exhibit a sufficient homology to the defined percentages.

[0031] The corresponding factor from *B. amyloliquefaciens* can be regarded as the closest prior art. The associated DNA sequences and amino acid sequences have been published in the NCBI databank of the National Institute of Health, USA (~~http://www.ncbi.nlm.nih.gov~~) under the entry number GenBank: AJ515542. RecA from *B. licheniformis* DSM 13 exhibits a homology on the amino acid level of 94% identity and on the nucleic acid level an identity agreement of 81.2%. Both comparisons emerge from the alignments of Figures 1 and 2, where the sequences of *B. amyloliquefaciens* are each presented in the second line.

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Please amend paragraph [0066] of the English-language translation of the Specification as follows:

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[0066] All of these genes are known *per se* and have been described for this sporulation phase. The *B. subtilis* gene *spoIVA* encodes for the phase-IV sporulation protein A that has been deposited in the databanks of Swiss-Prot (Geneva Bioinformatics (GeneBio) S.A., Geneva, Switzerland; <http://www.genebio.com/sprot.html>) and NCBI (see above) under the number Swiss-Prot: P35149. It plays a role in the formation of an intact spore hull and its assembly. The amino acid sequence of the associated factor *SpoIVA* is listed in SEQ ID NO. 8 of the present application, in fact as the translation of the previous DNA sequence produced by the PatentIn program. The associated nucleotide sequence can be found in the databank Subtilist Web server of the Institute Pasteur, Paris, France (<http://genolist.pasteur.fr/Subtilist/genome.cgi>) under the accession number BG10275 and is listed in the sequence protocol in SEQ ID NO. 7, in fact with the 200 nucleotides situated before the 5'-end and the 197 nucleotides situated behind the 3'-end. Here, irrespective of the fact that these boundary sequences are likely to comprise completely meaningful genetic information, in particular regulation elements or also segments of other genes, the complete nucleotide sequence from 1 to 1876 listed under SEQ ID NO. 7 is described according to the invention as the gene *spoIVA*. The encoded region extends from the positions 201 to 1679; the first codon, i.e. the positions 201 to 203 are not translated *in vivo* as leucine but rather as methionine.

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Please amend paragraph [0145] of the English-language translation of the Specification as follows:

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[0145] For the deletions, flanking regions from the chromosomal DNA were amplified with the oligonucleotides shown in Figure 3 and used for the construction of suitable deletion cartridges,

as is described below in more detail. They were created first in the *E. coli* vector pUCBM21. This is described under ~~http://seq.yeastgenome.org/vectordb/vector\_descrip/PUCBM21.html~~ (accepted on 14.1.2005) and is commercially available from Roche Diagnostics GmbH, Roche Applied Science, Sandhofer Str. 116, 68305 Mannheim (formally Boehringer). They were later cloned into the *Bacillus* vector pE194. This is ~~described under~~ ~~http://seq.yeastgenome.org/vectordb/vector\_descrip/PE194.html~~ (accepted on 14.1.2005) and is available from the American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209, USA (~~http://www.atcc.org~~).

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Please amend paragraphs [0165] through [0171] of the English-language translation of the Specification as follows:

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[0165] ~~Figure 1:~~ Figure 1: Amino acid sequence alignment of SEQ ID NO. 2 with closest prior art Rec factors.

The following meanings apply:

- [[1:]] 1: Factor RecA from [[B.]] *B. licheniformis* DSM 13 (~~SEQ ID NO: 2~~) (SEQ ID NO: 2)
- [[2:]] 2: Factor RecA from [[B.]] *B. amyloliquefaciens* (AJ515542 in NCBI) (SEQ ID NO: 34)
- [[3:]] 3: Factor RecA from [[B.]] *B. subtilis* (Z99112 in NCBI; Region 161035 to 162078) (SEQ ID NO: 36)
- [[4:]] 4: Factor RecE from [[B.]] *B. subtilis* (X521 32 in NCBI) (SEQ ID NO: 38)

[0166] ~~Figure 2:~~ Figure 2: Nucleic acid sequence alignment of SEQ ID NO. 1 with closest prior art *rec* genes.

The following meanings apply:

- [[1:]] 1: Gene *recA* from *B. licheniformis* DSM 13 (SEQ ID NO: 4) (SEQ ID NO: 1)  
[[2:]] 2: Gene *recA* from [[B:]] *B. amyloliquefaciens* (AJ515542 in NCBI) (SEQ ID NO: 33)  
[[3:]] 3: Gene *recA* from *B. subtilis* (Z99112 in NCBI; Region 161035 to 162078) (SEQ ID NO: 35)  
[[4:]] 4: Gene *recE* from *B. subtilis* (X521 32 in NCBI) (SEQ ID NO: 37)

[0167] ~~Figure 3:~~ Figure 3: Schematic Representation of the genetic organizations of the wild type as well as of the mutant-loci of *spoIV* (A) and *recA* (B), including the binding points for the primers listed under ~~SEQ ID NO:~~, SEQ ID NOs: 19 to 30.

[[A:]] A Functional inactivation (deletion) of *spoIV*, i.e. derivation of the strain *B. licheniformis* A.1 from *B. licheniformis* A (see example 2).

[[B:]] B Functional inactivation (deletion) of *recA*, i.e. derivation of the strain *B. licheniformis* A.2 from *B. licheniformis* A.1 (see example 2).

[0168] ~~Figure 4:~~ Figure 4: Genotypical investigation of the mutant strains A.1 and A.2 compared with the starting strain *B. licheniformis* A by means of PCR (A) and Southern analysis (B) (see example 3).

[0169] ~~Figure 5:~~ Figure 5: Graph of the living cell counts and the spore titer of the *B. licheniformis*-cultures. Each culture was examined in three parallel experiments. Each experiment was statistically validated by four determinations (see example 4).

The following meanings apply:

- Black, solid square: *B. licheniformis* A;  
open circle: *B. licheniformis* A.1 ( $\Delta spoIV$ );  
open triangle: *B. licheniformis* A.2 ( $\Delta spoIV$ ,  $\Delta recA$ );  
dashed line: Living cell counts  
solid line: spore titer

[0170] ~~Figure 6:~~ Figure 6: Growth curve of a culture of three *B. licheniformis* strains in minimal medium (see example 5).

[0171] ~~Figure 7:~~ Figure 7: Results of the UV tests

[[A]] A Graph of the survival rates after UV irradiation. Each culture was examined in three parallel experiments. Each experiment was statistically validated by double determinations (see example 6).

The following meanings apply:

Black, solid square: *B. licheniformis* A;

open circle: *B. licheniformis* A.1 ( $\Delta spoIV$ )

open triangle: *B. licheniformis* A.2 ( $\Delta spoIV$ ,  $\Delta recA$ ).

[[B]] B Qualitative UV test with exposure of the strains A.1 and A.2 on plates, which were half covered during the irradiation.

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